Hot Water Treatment and Insecticidal Coatings for Disinfesting Limes of Mealybugs (Homoptera: Pseudococcidae)

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J. Econ. Entomol. 93(3): 1017–1020 (2000)

ABSTRACT Hot water immersion and insecticidal coatings were tested to determine if they could be used to disinfest Persian limes, *Citrus latifolia* Tanaka, of the mealybug pests *Planococcus citri* Risso and *Pseudococcus odermatti* Miller & Williams. A 20-min 49°C hot water immersion treatment is effective in killing mealybugs and all other arthropods tested found externally on limes, or under the calyx. No insects or mites were found to survive after the 20-min hot water treatment. In this test, 7,200 limes were treated with 1,308 insects killed and zero survivors. Treatment at 49°C for 20 min did not significantly affect quality when treated fruit were compared with untreated control fruit. Four coatings were tested at a 3% rate: two petroleum-based oils (Ampol and Sunspray oil), a vegetable oil (natural oil), and a soap (Mpede). The coatings gave up to 94% kill (Ampol) of mealybugs, which is not sufficient to provide quarantine security. The coatings might be effective as a postharvest dip before shipment.

KEY WORDS mealybugs, Pseudococcidae, hot water immersion, insecticidal coatings, market quality, quarantine treatment

MEALYBUGS ARE MAJOR pests of many agricultural commodities. The hibiscus mealybug *Maconellicoccus hirsutus* (Green) currently damages crops on many Caribbean islands and threatens to reach the continental United States soon. Limes, *Citrus latifolia* Tanaka, which are imported into the United States from the Bahamas, are inspected for mealybug pests. A quarantine treatment is required to prevent these and other unwanted pests from spreading into the United States. When unidentifiable early stages, or actionable species of mealybugs are found, limes are fumigated with methyl bromide (Anonymous 1998). During cold weather, a higher dose is required that causes injury to the limes (Brian Weaver, Bahama Star, personal communication). Methyl bromide also is scheduled to be phased out, except for quarantine uses, because it is an ozone depleter. This phase out may increase the price of current methyl bromide treatments, making them less economical. For these reasons, alternative treatment for commodities infested with hitchiker pests such as mealybugs are required to prevent interruptions in commerce.

A number of treatment methods were considered for disinfesting limes of mealybugs. Hot water has been used to disinfest commodities of a variety of surface pests, including mealybugs (Lester et al. 1995; Hara et al. 1993, 1994, 1995, 1997). Based on this previous work with hot water to kill insects, we proposed that a hot water treatment of 46–57°C for 5–20 min would disinfest the surface of the limes of pests.

Coatings have been used successfully on grapes to disinfest them of mites (Hallman 1994), and tested on fruit to kill internal fruit fly larvae (Hallman et al. 1994, Hallman 1997). Several insecticidal coatings were tested, including vegetable and mineral oils, and insecticidal soap.

The objective of this study was to test hot water immersion and insecticidal coatings as quarantine treatments for mealybugs on limes. Both the coatings and the hot water treatment would fit in well as a rapid treatment on a packing line.

Materials and Methods

To resolve the issue of working with quarantined pests, shipments of limes were brought in from the Bahamas by the importer, treated on the dock with insecticidal coatings or hot water, examined for insect mortality, then returned to the Bahamas for disposal.

Hot Water. A 200-liter stainless steel tank of water was heated to the desired temperature with a propane gas heater. Preliminary tests indicated that limes would tolerate 49°C for up to 15 min without showing damage, so this temperature was used for the dose-mortality study. Limes with feral mealybug populations were held in nylon mesh bags and dipped in groups of 120 fruit at times of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, and 16 min in hot water at 49 ± 0.5°C. This test was repeated five times with different shipments of limes. More than 6,000 limes were used in the dose-mortality tests. The water temperature was monitored with a national standards traceable thermometer. After removal from the hot water, the limes were hydrocooled for 10 min in a water tank held at 25 ± 2°C. The limes were then placed in plastic bins (30 by 45 by 15 cm i.d.) that were placed in larger pans of water with detergent soap added to prevent escape of mealybugs. The limes were held for 2 or 3 d at 24 ± 2°C, and then examined under a stereo microscope (10 or 20×) and live and dead insects present on the surface and under the calyx of the limes were counted. Nymphs and adult mealybugs were counted. Insects that did not move when probed were recorded as dead. One control group of limes (n = 80) was held without treatment and examined for insects at the same time as the treated limes. Data were analyzed with regression and probit anlysis (Tablecurve; Jandel Scientific 1994).

After a potential treatment time of 20 min was identified from the dose-mortality tests, a large-scale test was initiated. Limes were dipped in groups of 120 in nylon mesh bags in hot water for 20 min at 49°C. A control group of 80 limes was held without treatment. All fruit were examined for dead and live insects (both nymphs and adults) 2 d after treatment. This large-scale test was conducted when fruit were available in groups of 1,200 fruit. The test was continued for six replications at which time a cumulative total of >1,000 mealybugs had been treated with no survivors. All stages of mealybugs found on treatments and controls were sent to the Systematic Entomology Laboratory of the USDA for identification.

Coatings. Coatings were applied at a 3% (vol:vol) rate in ≈10 liters of water. Groups of 60 limes were dipped in coatings for 10 min, rinsed 10 min in tap water, then held for 2 or 3 d before evaluation for live and dead nymphs and adults counted as described in the previous experiment. Two different petroleum based oils (AMPOL, Caltex Australia, Sydney, New South Wales; and Sunspray Ultra-Fine Spray Oil, Sunoco, Philadelphia, PA), a vegetable oil (Natural Organic oil, Custom Chemicides, Fresno, CA), and a soap (Mpede, Mycogen, San Diego, CA) were tested. Controls were prepared as in the previous experiment. This experiment was repeated seven (vegetable oil and Sunspray oil) or eight (soap and AMPOL) times. Data were analyzed with analysis of variance (ANOVA) and Waller–Duncan K-ratio test (PROC GLM, SAS Institute 1988).

Phytoxicity. All fruit were weighed, and groups of 40 limes were immersed for 10, 15, or 20 min in water at 46, 49, and 52°C. All fruit were cooled in water at ≈25 ± 2°C for 10 min after hot water immersion. One group of fruit was not treated and served as a control. When storage at 22°C was complete after 8 d, all fruit were weighed a second time and rated for the percentage of surface injury with a 12-point visual acuity scale (Horsfall and Barratt 1945). Injury was defined as the development of a brown or black discoloration on the fruit surface. Firmness of each unpeeled fruit was measured with a Force Five multicapacity force gauge (Wagner Instruments, Greenwich, CT) mounted on a Wagner FTC 100 test stand; resistance was recorded after a compression of 3 mm. Surface color was recorded with a Minolta CR-200 chroma meter (Minolta, Ramsey, NJ) to a standard white reflective plate and recording in the L*C*h° color system (lightness, chroma, and hue angle, respectively).

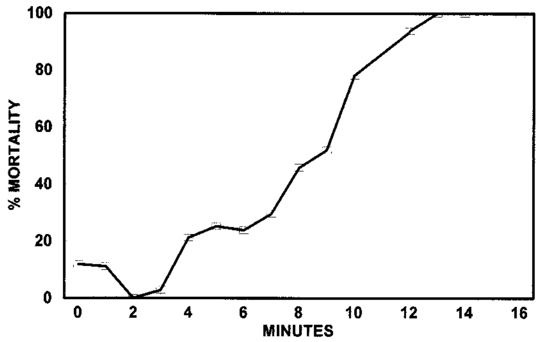

Fig. 1. Mealybug (Homoptera: Pseudococcidae) mortality on limes dipped in 49°C water.

Measurements were taken across an area ≈50 mm^2 with diffuse illumination at a viewing angle of 0° under Commission Internationale de l'Eclairage illuminant C conditions. Eight fruit per treatment were juiced with a hand juicer. After first measuring the pH of the juice with a Corning combination electrode (Thomas Scientific, Swedesboro, NJ), 10 ml was titrated with 0.1 M NaOH to a pH of 8.1 for calculation of the titratable acidity expressed as anhydrous citric acid. A refractive index (Fisher Abbe refractometer, Fisher, Pittsburgh, PA) also was determined from the juice for calculation of the percentage of soluble solids. The concentration of ascorbic acid was determined by the 2,6-dichloroindophenol titrimetric method (AOAC 1984). This experiment was repeated four times. Data were analyzed with ANOVA and means separation by the Ryan–Einot–Gabriel–Welsh multiple F test in SAS (SAS Institute 1988).

Results

Hot Water. Hot water treatment of limes showed little effect on mortality until 5 min, then mortality increased until no survivors were recovered after 12 min of 49°C (Fig. 1). Linear regression gave 99.9968% mortality (probit 9) predictions at 13 min (Fig. 2). A

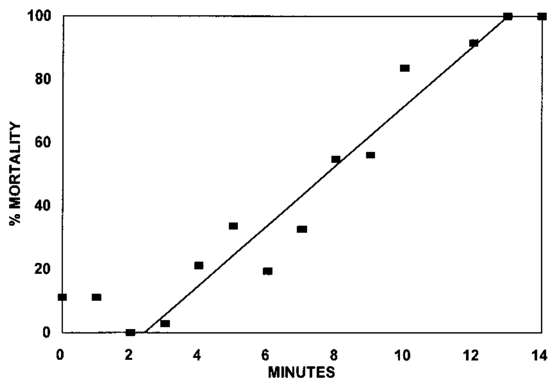

Fig. 2. Regression of mealybug (Homoptera: Pseudococcidae) mortality on limes dipped in 49°C water ($y = 9.44x - 22.74$, $r^2 = 0.93$, $n = 15$, $F = 158.77$).

Table 1. Mortality of mealybugs on limes treated with different coatings

Coating	Mortality, mean ± SE
AMPOL oil	94.0 ± 6.0a
Natural oil	65.3 ± 10.0b
Sunspray oil	57.0 ± 17.0bc
Mpede	30.4 ± 10.0c
Control	2.2 ± 2.0d

Means followed by the same letter are not significantly different (Waller–Duncan k-ratio test, F = 12.91; df = 12, 22; $P < 0.05$ [SAS Institute 1991]).

linear regression model fit the data with an r^2 of 94.6% (Fig. 2; data points beyond the first 100% mortality data point excluded because mortality cannot go above 100%, and the first two data points excluded). A large scale test at 20 min was initiated and batches of 1,200 limes dipped and examined for live and dead insects. In this test, 7,200 limes were treated with 1,308 insects killed and zero survivors. The mealybugs were immatures and adults of both *Planococcus citri* Risso (50%) and *Pseudococcus odermatti* Miller & Williams (50%).

Coatings. Using ANOVA (Waller–Duncan k-ratio test), we found significant differences among the treatment means (Table 1). The soap, Sunspray oil, and vegetable oil coatings were ineffective with mortalities of 30, 57, and 65%, respectively. The AMPOL oil coating was more effective providing 94% mortality. Significantly lower numbers of dead and living insects were recovered in the treatments than the control (Table 2). This implies that the coatings repelled the insects and caused them to leave the fruit, or caused them to drop off when the treatments were applied as a dip.

Phytotoxicity. Treatment temperature and the duration of the immersion significantly affected the fruit quality. Treatment at 52°C was significantly more damaging than treatment at 46 or 49°C (Table 3). At 52°C injury and weight loss were greatest, and the fruit were less firm. Fruit color was lighter, more intense, and less green, and the pH of the juice and acidity increased slightly. The effect of treatment time was less significant. Firmness and juice characteristics were not affected by time of treatment between 10 and 20 min at these temperatures. Injury and weight loss were greater after a 20-min treatment, and surface color was lighter, more intense, and less green with increasing time. Treatment at 49°C for 20 min did not significantly affect quality when treated fruit were compared with untreated control fruit.

Table 2. Living and dead mealybugs recovered on limes after treatment with different coatings

Coating	No. of mealybugs mean ± SE (per 60 limes)
Control	20.6 ± 8.1a
Natural oil, 3%	5.5 ± 1.7b
Mpede, 3%	5.4 ± 1.5b
Sunspray oil, 3%	4.5 ± 2.4b
AMPOL oil, 3%	3.6 ± 1.8b

Means followed by the same letter are not significantly different (Waller-Duncan K-ratio test, F = 2.79; df = 12, 26; $P < 0.05$ [SAS Institute 1991]).

Discussion

No surviving mealybugs or other arthropods were found after the 20-min 49°C hot water immersion treatment. This included small numbers of Coleoptera, Hymenoptera, Lepidoptera, Thysanoptera, and unidentified mites found externally or under the calyx. Water temperatures of 49°C have been shown to kill external insect pests in a very short time, <10 min (Sharp 1994). The insects on the limes survived hot water longer than might be predicted from tests done with insects directly immersed in hot water. This was because the water did not penetrate underneath the calyx of many fruits and the heating of this area is indirect, and therefore slower. Researchers in Hawaii found similar results with hot water immersion to treat cut flowers for scale insect infestations, aphids, and mealybugs (Hara et al. 1993, 1994, 1995, 1997). They found that a 10-min immersion in 49°C hot water achieved 100% mortality of all stages of *Pseudaulacaspis cockerelli* (Cooley) and *Coccus viridis* (Green). They found that a 12-min immersion in 49°C water eliminated 95% of ants, aphids, and mealybugs on red ginger flowers, *Alpinia purpurata* (Vieill.) K. Schum. Lester et al. (1995) found that longtailed mealybug, *Pseudococcus longispinus* (Targioni-Tozetti), required an estimated 19 min to reach 99% mortality on persimmons, *Diospyros kaki* L., dipped in 49°C hot water.

Thus, the treatment time of 20 min is very conservative and is based on the theoretical prediction of survivors if large numbers of insects are treated. The data show that a treatment >15 min is unnecessary, but a 20-min treatment will give an added safety factor without affecting fruit quality.

It has been shown that grapefruits will not tolerate treatments of 20 min with 49°C hot water without showing damage (Sharp 1985), but limes in this study tolerated a 20-min treatment with no loss in quality. It is possible that the immediate hydrocooling of the limes was a factor in maintaining market quality.

The coatings gave up to 94% kill of mealybugs, but this is insufficient to provide quarantine security. As with hot water treatment, the calyx of the limes provided protection to the mealybugs and prevented a higher treatment mortality. If the coatings were applied as a postharvest dip before shipment, they would reduce the numbers of pests, and make it less likely that an inspector would find actionable pests at the destination, but they do not provide quarantine security.

The treatment of 20 min at 49°C gave quarantine security for limes infested with mealybugs and did not significantly affect fruit quality. Coatings reduced the populations of insects but did not achieve probit nine quarantine security.

Table 3. Quality of limes immersed in water at different temperatures and times

Effects	Surface injured %	Weight loss %	Firmness *n*	Surface color			Juice characteristics			
				L*	C*	h°	pH	Acids %	Solids %	Ascorbate ppm
Temp,°C										
46	0.5b	6.7ab	26.6a	54.1b	43.4b	116.2a	2.25b	6.86b	8.80a	16.00b
49	0.5b	6.4b	26.1ab	54.8b	43.9b	115.5b	2.27b	6.94ab	8.72a	15.83b
52	3.5a	7.0a	25.3b	57.3a	46.1a	112.3c	2.33a	7.05a	8.78a	17.21a
Time, min										
10	0.6b	6.4b	26.3a	54.6b	43.6b	115.4a	2.29a	6.96a	8.84a	17.03a
15	1.1b	6.4b	26.3a	55.8a	44.8a	114.6b	2.29a	6.90a	8.74a	15.98b
20	3.0a	7.3a	25.5a	55.8a	44.9a	114.0c	2.28a	6.99a	8.73a	16.03b
Treatments										
Untreated	0.4a	6.3a	27.0a	51.9a	39.9a	117.7a	2.32a	6.88a	8.86a	17.58a
20 min, 49°C	0.6a	6.5a	25.6a	55.6a	44.6a	114.8a	2.28a	6.86a	8.65a	15.30a

Within columns of a particular effect, means followed by the same letter were not significantly different at $P = 0.05$ based on ANOVA and means separation by the Ryan–Einot–Gabriel–Welsh multiple *F* test (*t*-test for "treatments") in S AS. Means of 480 fruit (time and temperature) or 160 fruit (treatments). Color: L*, higher values are lighter; C*, higher values are more intense; h°, 0° = red-purple, 90° = yellow, 180° = bluish green, 270° = blue. For treatments; L*, C*, and h° are significant at $P = 0.12$, 0.07, and 0.10, respectively.

Acknowledgments

We thank P. Mendez, W. Montgomery, and E. Schnell (USDA-ARS) for their assistance. We thank D. R. Miller (USDA-ARS-SEL) for identifying the mealybugs used in this study. We thank G. Hallman (USDA-ARS, Weslaco, TX), A. Hara (University of Hawaii at Manoa, Hilo), R. Paull (University of Hawaii at Manoa, Hilo), and V. Yokoyama (USDA-ARS, Fresno, CA) for providing constructive reviews of the manuscript. We thank Bahama Star Ltd. for supporting this study. We thank APHIS-PPQ for their cooperation in facilitating this work.

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Received for publication 6 October 1999; accepted 28 February 2000.